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Effect of Feeding Protected Safflower Oil on Yield, Composition, Flavor, and Oxidative Stability of Milk

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ABSTRACT

Four Holstein cows were fed 800 g of safflower oil:casein:formaldehyde per day for 16 wk as supplement to a hay:concentrate diet. Four control Holstein cows were fed only the hay:concentrate diet. The safflower oil in the supplement was protected from hydrogenation in the rumen. The linoleic acid content of the milk fat was increased from a mean of 2.7% for nonsupplemented cows to 13.3% for supplemented cows. Recovery in milk fat of protected linoleic acid was 22%. Milk, fat, and protein yields and fat and protein percentages were not affected by the supplementation. No health or feeding problems were observed during the supplementation with the safflower oil:casein:formaldehyde material. Off-flavors, predominately of an oxidized nature, readily developed in milk containing high linoleic acid. Supplementation of the cows with α-tocopheryl acetate or the direct addition of α-tocopherol to the milk effectively prevented development of oxidized off-flavors.

INTRODUCTION

Experiments have shown the feasibility of modifying the fatty acid composition of milk fat by feeding cows a diet containing safflower oil:casein:formaldehyde (SOC-F) (7, 8, 11, 14, 18, 26, 27). Fatty acid composition of adipose tissue can be modified from a saturated to a more unsaturated fatty acid content by feeding ruminant animals a "protected" vegetable oil (10, 14, 28).

Use of the Scott fat protection principle (28) has shown that milk fat containing 35% linoleic acid can be produced (26). The percentage of fat in the milk also increased significantly. An intake of 200 to 250 g of "protected" linoleic acid per day per cow resulted in 10% linoleic acid in the milk fat (14).

Previous experiments at this research facility consisted of treatments of 7 or fewer days. The purpose of this experiment was to determine the effect of feeding one amount of protected safflower oil for 16 wk on production and composition of the milk, general health of the cows, blood lipid profile, and adipose tissue changes. The development of oxidized flavors in milk containing high polyunsaturated fatty acids was also studied along with methods of prevention.

PROCEDURE

Eight Holstein cows ranging from 471 to 631 kg in body weight and from 60 to 170 days of lactation (first to fourth lactation) were divided into four pairs on similar digestible energy (DE) requirements. Cows within pairs were assigned randomly to the two rations. Each cow received baled, medium-quality alfalfa:orchardgrass hay to meet calculated DE requirements for maintenance and concentrate to meet calculated DE requirements for milk (24). Treated cows received the same ration, but 800 g of SOC-F replaced an equal weight of concentrate. Therefore, each cow received the SOC-F at slightly different ratios of total intake. Analyses of SOC-F indicated that the composition was 68.5% oil (chloroform:methanol extraction), 28.5% crude protein (Kjeldahl), .5% water (oven), .5% formaldehyde, and 2.0% unidentified material, probably partly mineral contained in the sodium caseinate. Formalde-

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hyde was determined by a modification of the method reported by Swain et al. (32) involving hydrolysis with phosphoric acid and distillation, followed by spectrophotometric measurement with chromotropic acid with the conditions of MacFadyen (21). The hay and concentrate were analyzed for nitrogen (Kjeldahl), dry matter (oven), and fat content (chloroform:methanol extraction). The duration of the experiment was 16 wk, and cows were weighed every 4 wk.

Two consecutive milkings were sampled weekly and composited for each cow for analysis for fat (Foss Milko-Tester), protein (Kjeldahl), solids-not-fat by the Watson lactometer method (SNF), and fatty acid composition of milk fat. Lipids were extracted from milk samples by the method of Storry and Millard (31). Methyl esters of the fatty acids were prepared by the method of Christopherson and Glass (9). The composition of methyl esters was determined by programmed gas liquid chromatography with 10% ethylene glycol succinate-methyl silcone polymer (EGSS-X) on Gas-Chrom P (100/120 mesh) in a 6-mm diameter glass column.

Blood samples from jugular vein were taken at weekly intervals. Milk, plasma, and total cholesterol were determined weekly on individual samples from each cow by the method of Sobel and Mayer (29). Composition of plasma for triglycerides (33), nonesterified fatty acids (1), and lipid fatty acids was determined. A biopsy of tailhead adipose tissue was obtained at biweekly intervals and each sample analyzed for fatty acid composition.

The effect of vitamin E supplementation of the control and SOC-F supplemented cows upon the development of oxidized flavor in the milk was determined after the cows had been on the lipid supplement for 2 mo. Both control and SOC-F fed cows received 5 g of α-tocopheryl acetate per day for 7 days. Milk samples were taken before the supplementation, at day 5 during the vitamin E feeding, and 2 days after vitamin E treatment was stopped. α-Tocoperol also was added directly to freshly drawn milk to control oxidized flavor. Tocopherol was dispersed in Triton X-100 and diluted with water to a concentration of 1 mg/ml, and varying amounts were added to the milk (20).

Vitamin E was determined in the milk fat by the method of Erickson and Dunkley (16). After 2 days of refrigerated storage, milks were evaluated for spontaneous oxidized flavor and copper-induced (.1 ppm Cu added) oxidized flavor; the thiobarbituric acid reaction as described by King was used (19). Flavor also was evaluated organoleptically by a three-judge panel.

Analyses of variance were a whole plot containing the correction factor, treatment, and error A. The split-plot contained week and treatment-by-time interaction and error B. A 1-wk preliminary period of production criteria was a covariate without any changes in conclusions so the analysis is not presented.

RESULTS AND DISCUSSION

Intake and Feed Composition

Mean daily dry matter intake and dry matter as a percentage of body weight did not differ ($P>.05$) between control and treatment groups (Table 1). Intakes of DE and fat were higher for SOC-F supplemented cows as a result of the higher fat content of SOC-F supplement. Protein intake was similar for both treatment groups. Intake of DE was estimated 107% of National Research Council (24) requirements for the control and 109% for the SOC-F treatment groups. Protein intake was >110% of the estimated requirements. Body weight did not change significantly for any of the experimental animals. Health of animals appeared to be normal.

Milk Yield and Composition

Table 2 gives the treatment means for milk yield, fat-corrected milk (FCM), and milk constituents. No differences were significant ($P>.05$) for yield of milk, milk fat, milk protein, and milk SNF (solids-not-fat). Fat, protein, SNF, and cholesterol concentration in milk were not different ($P>.05$) between treatments. Previous work at this research center (14, 26) and elsewhere (25) has shown that the percentage of fat in milk or the milk fat yield or both increase when cows are supplemented with safflower oil protected with casein and formaldehyde. In this experiment, there were increases ($P>.05$) in milk yield (7%), FCM (5%), percentage of fat (2%), fat yield (7%), percentage of crude protein (3%), protein yield (5%), percentage of SNF (3%), and SNF yield (5%)

TABLE 1. Mean intake of various diet components for the two treatment groups of four cows.

	Control cows	SOC-F[a] cows	Standard error
Dry matter (kg/day per cow)	16.0	16.1	1.51
% of body weight	2.94	2.90	.25
Digestible energy total (Mcal/day)	50.9	53.3	...
Concentrate	36.6	31.7	4.96
SOC-F	...	5.7	...
Hay	14.3	15.9	.70
Fat total (g/day)	609	1117	...
Concentrate	415	359	56
SOC-F	...	542	...
Hay	194	216	9.5
% Fat of total intake (w/w)	3.81	6.94	...
Protein total (g/day)	2708	2818	...
Concentrate	1687	1458	228
SOC-F	...	225	...
Hay	1021	1135	50

[a]Safflower oil:casein:formaldehyde.

for SOC-F supplemented cows over similar items for control cows. The previous reports showing increases in fat percentage in milk (14, 26) were based on shorter term feeding (5 to 7 days) and a higher intake (1500 to 3200 g) for SOC-F than in this experiment. Another difference in this experiment was that the control was not receiving high unprotected fat in the rumen. Australian work (25) suggested that the higher milk fat content was compensated by a reduced SNF content.

Daily production of milk, milk fat, and milk protein decreased ($P<.05$) with time during the experimental period for both treatment groups. This decrease was attributed to the normal decline of advancing lactation. The decrease in milk production for both control and SOC-F fed cows during the experiment was 16% or 1% per wk, which is less than the expected 2% per wk. Weekly means of fat production for each treatment group were different ($P<.05$) during the experimental period. Weekly treatment means for percentage of fat were variable but not significantly different ($P>.05$).

The fatty acid distribution (weight %) in the concentrate, hay, and SOC-F parts of the feed is shown in Table 3. The safflower oil assayed 74.9% linoleic acid. All feeds were high in unsaturated acids. However, only the SOC-F part was protected from rumen hydrogenation.

Mean daily intake and production in milk fat of each fatty acid for both treatment groups are

TABLE 2. Milk yield and composition of control and safflower oil:casein:formaldehyde supplemented cows.

	Control	SOC-F	Standard error
Milk (kg/day)	22.5	23.0	2.42
Milk FCM[a] (kg/day)	20.4	21.4	2.28
Milk fat (%)	3.4	3.5	.13
(g/day)	761	812	89.8
Milk protein (%)	3.3	3.4	.11
(g/day)	749	784	81.0
Milk solids-not-fat (%)	8.8	9.1	.68
(g/day)	1982	2075	304.6
Milk cholesterol (mg/100 ml)	14.4	13.5	.44

[a]FCM = fat-corrected milk.

TABLE 3. Fatty acid distribution (weight %) in the fat of the three types of feeds given to cows.[a]

Fatty acid	Concentrate	Hay	SOC-F[b]
C12:0		1.0	.2
C14:0		.9	
C16:0	12.4	19.1	8.4
C16:1		3.0	.2
C18:0	1.8	3.4	2.4
C18:1	21.8	6.6	13.1
C18:2	58.7	24.3	74.9
C18:3	4.9	40.4	.5
Others		1.5	.3

[a]Laboratory analyses of feeds fed.

[b]Safflower oil:casein:formaldehyde.

in Table 4. SOC-F increased ($P<.01$) daily production of C18:2 and decreased ($P<.05$) daily production of C14:1 and C16:1. The control group had an average daily intake of 507 g of C18's and produced 297 g in the milk for a 59% recovery whereas the SOC-F supplemented cows consumed 967 g of C18's and produced 480 g for a 50% recovery. Fatty acids below C18 showed a net gain in yield over intake for both treatment groups as expected because these acids are largely synthesized *de novo* in the mammary gland from acetate and β-hydroxybutyrate (12). Ruminal synthesis of fatty acids can amount to 140 g/day in the lactating dairy cow (17). The control group had five times as much of these lower acids in milk fat as in feed whereas the SOC-F group had two and one-half times as much. These results suggest that when higher amounts of protected C18 acids are included in the diet, there is less synthesis of fatty acid below C18.

Responses have been similar when unprotected vegetable oils rich in C18:2 or C18:3 were fed to lactating cows (22, 27). One-half of the C18's fed to the SOC-F group were derived from the SOC-F ingested and, therefore, theoretically were protected from rumen hydrolysis and hydrogenation. The subsequent transfer of increased amounts of C18 acids into the SOC-F milk was compensated for by decreased amounts of C6 to C16 fatty acids. Thus, all C6 to C16 acids in SOC-F milk declined about 30% (Table 4), which was a mean net decrease of 130 g/day in production. The C18 acids increased about 62%, which was a mean net increase of 183 g/day in the SOC-F milk. The proportion of C18's in the milk fat of the control group was 29% and increased to 59% in the SOC-F fed group. Other studies involving the use of protected lipids have shown that a decrease in the production of the lower fatty acids accompanies the increase in transfer of C18 acids into the milk fat (11, 18). If all milk fat linoleic acid were derived from the diet, then the recovery of linoleic acid was 7% for the control and 15% for SOC-F supplemented group. Recovery was calculated by taking the milk linoleic acid produced as a percent of linoleic acid consumed. The recovery of the protected linoleic acid fed was 22% when all the increase in the milk over the control is attributed to linoleic acid consumed via the SOC-F. Previous experience (7, 25) has shown that the formaldehyde protection of safflower oil allows a 15 to 25% recovery of linoleic acid.

Changes in major C18 acids have been reported (7, 14) when protected safflower oil was fed. Figure 1 shows that the weekly mean linoleic acid content of milk fat for the SOC-F group was about four times higher after the 2nd wk of the experiment.

The polyunsaturated fat in milk can be increased greatly and maintained over a relatively long period by feeding protected safflower oil to cows. Previous experiments in

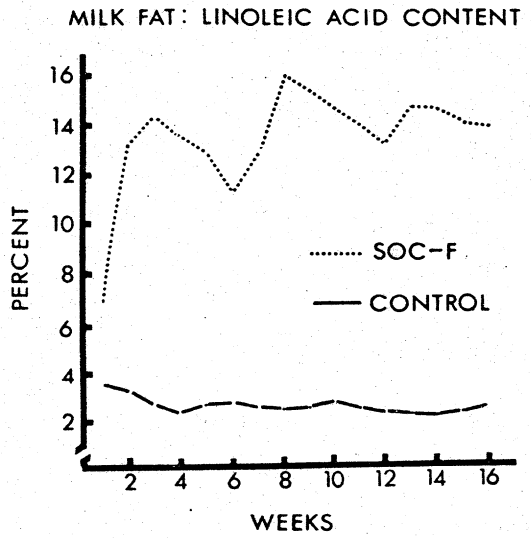

FIG. 1. Percentage of linoleic acid in the milk fat for the cows supplemented with safflower oil:casein:formaldehyde (SOC-F) and the control cows.

TABLE 4. Mean intake and production of fatty acids (g/day per cow) for the safflower oil:casein:formaldehyde (SOC-F) supplemented cows and the control cows.

Fatty acid	Control				SOC-F					Standard error of a mean for milk fat
	Intake			Milk fat	Intake				Milk fat	
	Concentrate	Hay	Total		Concentrate	Hay	SOC-F	Total		
C4:0				17.3					18.1	2.93
C6:0				13.5					11.8	2.21
C8:0				8.4					6.9	1.39
C10:0				20.0					15.0	3.11
C12:0		1.9	1.9	24.9		2.2	1.1	3.3	17.7	3.44
C14:0		1.7	1.7	89.9		1.9		1.9	63.5	10.21
C14:1				11.8*					6.9*	1.36
C15:0				8.3					5.8	.88
C16:0	51.5	37.1	88.6	235.7	44.5	41.2	45.5	131.2	161.3	25.64
C16:1		5.8	5.8	19.3*		6.5	1.1	7.6	12.4*	1.86
C17:0				5.1					3.8	.46
C18:0	7.5	6.6	14.1	79.6	6.5	7.4	13.0	26.9	116.8	12.43
C18:1	90.5	12.8	103.3	189.0	78.2	14.2	71.0	163.4	244.2	19.12
C18:2	243.7	47.2	290.9	20.4**	210.7	52.5	405.9	669.1	107.3**	10.03
C18:3	20.3	78.5	98.8	7.9	17.6	87.2	2.7	107.5	11.3	1.09
Others		2.9	2.9	10.8		3.3	1.6	4.9	8.6	.99

*Means different between treatments ($P<.05$).

**Means different between treatments ($P<.01$).

which protected safflower oil was fed to cows (7, 8, 11, 14, 18, 26, 27) and to a goat (4) were of relatively short duration. Our present experiment indicates that the milk fat changes can be maintained over longer time and that there are no apparent effects upon health of the animals.

Blood and Plasma Components

Ratios of fatty acids in plasma circulating fat displayed trends similar to those of milk fatty acids. Linoleic acid in plasma was higher ($P<.01$) for the SOC-F fed group (Fig. 2). The correlation of milk fatty acids and plasma fatty acids has been shown many times (2, 5, 6, 23, 30, 34).

Plasma cholesterol was higher ($P<.05$) for the SOC-F supplemented group (Fig. 2) than for the control group. It increased ($P<.01$) during the experimental period, and control group cholesterol also increased during that time but not as much because interaction of treatment × week was significant ($P<.05$). We have observed an effect of stage of lactation on cholesterol which agrees with the recent report of Arave et al. (3). The increase of blood cholesterol may result from the higher total fat intake of the SOC-F cows or from the high polyunsaturated fat absorbed in the lower tract. Because fat intake and protected polyunsaturated fats are confounded, a discussion of the cause of the cholesterol rise can only be speculative. Plasma triglycerides and nonesterified fatty acids were higher ($P<.05$) for the SOC-F cows. However, the variation in these measurements was large. The increase in triglycerides and nonesterified fatty acids may represent the greater transfer of dietary lipid into the blood.

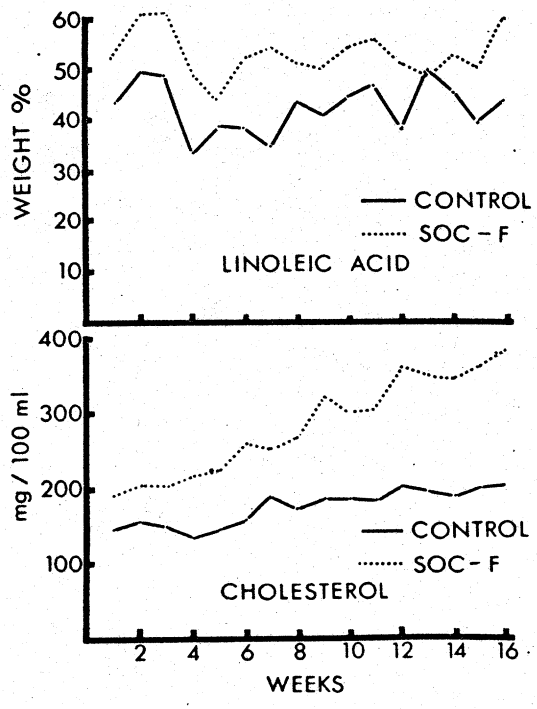

FIG. 2. Weekly treatment mean percentage of linoleic acid in plasma lipid and plasma cholesterol concentration of control cows and of cows supplemented with safflower oil:casein:formaldehyde (SOC-F).

Body Fat Composition

Tailhead adipose tissue indicated a change in fatty acid composition during the experimental period for the SOC-F fed group. The linoleic acid content was greater for the SOC-F group than for the control group and continued to increase throughout the experiment (Fig. 3). In a previous study (14), the tailhead linoleic acid increased from 2 to 6% of the total fatty acids in 5 wk when cows were fed increasing protected safflower oil during succeeding weekly intervals. Feeding SOC-F to growing steers (230 kg) increased the linoleic acid content of adipose tissue, but feeding SOC-F to finishing steers (474 kg) increased the linoleic content only slightly (13).

Oxidized Flavor in Milk

Polyunsaturated fatty acids are readily oxidizable at the double bonds, and milk contain-

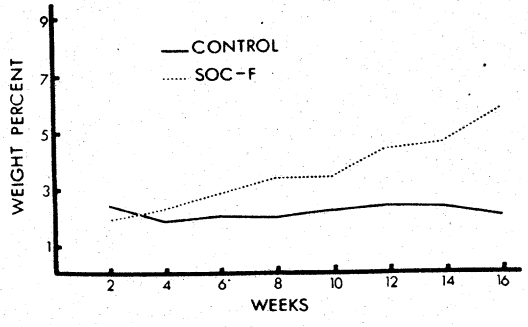

FIG. 3. Linoleic acid in the tailhead adipose tissue of cows supplemented with safflower oil:casein:formaldehyde (SOC-F) and in that of control cows.

ing increased amounts of these acids quickly develops a strong off-flavor, which is predominantly of an oxidized nature. We attempted to control the development of these oxidized off-flavors 1) by supplementing the cow's diet with α-tocopheryl acetate and 2) by adding α-tocopherol directly to the milk.

The rate of development of spontaneous and copper-induced oxidized flavor in milk from cows fed SOC-F is shown in Table 5. The milks were initially free of flavor defects, and the control milk (2.7% linoleic acid in milk fat) showed a high degree of stability. The milks were free of significant spontaneous or copper-induced oxidized flavor after 4 days of storage. Spontaneous oxidized flavor developed readily in milks (13.6% linoleic acid in milk fat) from two of the four SOC-F supplemented cows. Copper-treated milks from all SOC-F cows were oxidized strongly after only 24 h of storage.

The fact that freshly drawn milk from SOC-F fed cows showed negligible oxidized flavor raised the possibility of control of the development of off-flavors by the addition of antioxidants to the milk. Tocopheryl acetate (5 g/day) was fed for 7 days to both control and SOC-F supplemented cows to increase natural antioxidant, vitamin E in the milk (Fig. 4). Although this vitamin E supplementation. results in the transfer of increased amounts of vitamin E into the milk of control cows (+50%), a surprisingly larger amount of vitamin E was transferred into the polyunsaturated milk. Vitamin E in the polyunsaturated milk showed a 200% increase. Such an increase suggests that the transfer of dietary vitamin E was facilitated markedly by the increased amounts of lipid and cholesterol that were absorbed in the cows fed protected lipid (Fig. 2 and 3). Milk samples taken 2 days after vitamin E supplementation was stopped, still showed this large differential response, and vitamin E content of the polyunsaturated milk was about two times that in control milk.

Oxidized flavor was assessed by taste panel evaluation and thiobarbituric acid determination on the vitamin E supplemented milks after 2 days of refrigerated storage (Fig. 5). The pre-vitamin E supplementation milks from SOC-F fed cows showed much greater oxidized flavor than that of control milks. The presence of vitamin E in the milk effectively lowered the flavor score, but only spontaneous oxidized flavor was controlled (Fig. 5c).

Results of the direct addition of an antioxidant to control the off-flavor in polyunsaturated milk are in Table 6. Direct addition of emulsified tocopherol to about 75 μg/g fat controlled both spontaneous and copper-in-

TABLE 5. Rate of development of spontaneous and copper-induced oxidized flavor in milk from cows supplemented with safflower oil:casein:formaldehyde particles.[a]

Days storage	Optical density[b]		Flavor score[c]	
	Control	+Cu	Control	+Cu
		Untreated cows		
0	.017	...	0	...
1	.021	.023	0	.5
2	.031	.041	.13	.25
4	.046	.048	.13	.75
		Oil supplemented cows		
0	.020	...	...	...
1	.034	.046	.63	3.25
2	.048	.095	1.38	3.75
4	.061	.099	1.75	3.50

[a]Average of four cows in each group.

[b]Measured by the thiobarbituric acid reaction.

[c]Flavor scores are taste panel averages: 0 = none; 1 = questionable; 2 = slight but detectable; 3 = definite; 4 = very strong.

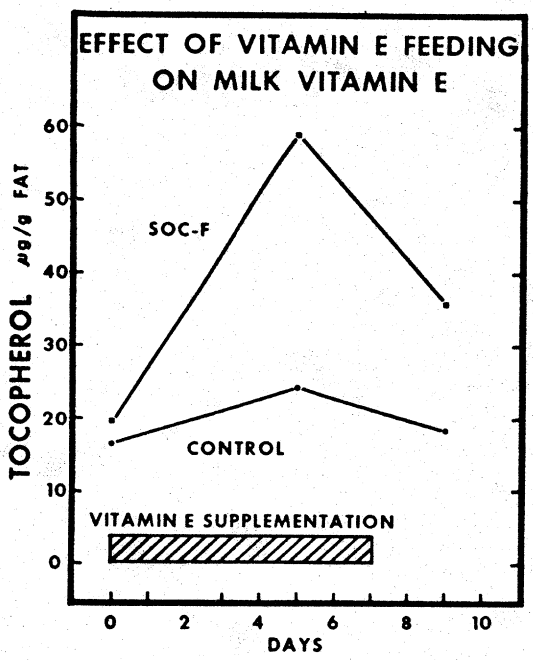

FIG. 4. Effect of vitamin E feeding to control and safflower oil:casein:formaldehyde (SOC-F) cows on milk vitamin E levels.

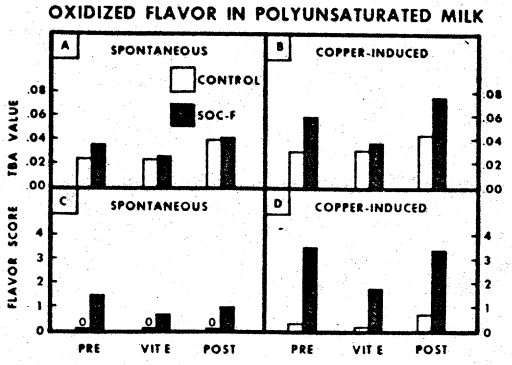

FIG. 5. Effect of vitamin E supplementation on the development of spontaneous and copper-induced oxidized flavor in milk before supplementation (PRE), at day 5 during vitamin E feeding (VIT E), and 2 days after vitamin E treatment was stopped (POST) on control and on safflower oil:casein:formaldehyde (SOC-F) fed cows.

duced oxidized flavor after either 2 or 5 days of storage. We have reported that the direct addition of α-tocopherol successfully prevented the development of off-flavors in milk containing 8% linoleic acid (15).

Direct addition of α-tocopherol to milk was more effective than feeding α-tocopheryl acetate to cows in achieving levels of vitamin E that would control the development of oxidized off-flavors. The transfers of vitamin E

TABLE 6. Effect of direct addition to milk of emulsified tocopherol on spontaneous and copper-induced oxidized flavor.[a]

Sample no.	Toc. added (µg/g fat)	Optical density[b]		Flavor score	
		2 days	5 days	2 days	5 days
		Samples without added copper			
1	0	.029	.029	0	1.0
2	30	.027	.025	0	0
3	60	.022	.033	0	0
4	120	.027	.030	0	0
5	240	.029	.032	0	0
		Samples with 1/10 ppm added copper			
6	0	.053	.062	3.0	3.5
7	30	.032	.039	0	1.5
8	60	.034	.041	0	.5
9	120	.032	.037	0	.5
10	240	.032	.039	0	.5

[a]Milk was a blend from four cows receiving oil supplement. Tocopherol content of blend before the addition of α-tocopherol was 25.1 µg/g fat.

[b]Measured by the thiobarbituric acid reaction.

[c]Flavor scores are taste panel averages: 0 = none; 1 = questionable; 2 = slight but detectable; 3 = definite; 4 = very strong.

achieved by feeding were relatively inefficient although milk contents were much higher in the presence of the protected lipid diet than in the control diet. Only spontaneous oxidized flavor was controlled in milk from vitamin E supplemented cows whereas both spontaneous and copper-induced oxidized off-flavors were controlled by the direct addition of vitamin E. The control of both spontaneous and copper-induced oxidized off-flavors by the direct addition of α-tocopherol, which is in contrast to control of only spontaneous off-flavors by α-tocopheryl acetate feeding, was probably due to higher milk vitamin E achieved by direct addition. Our experiments show that oxidized off-flavors can be controlled readily and effectively in polyunsaturated milk with the use of antioxidants.

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